PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference MJ/GK/LLH/PAT/8117441/PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. **PCT/SG2004/000194**	International filing date *(day/month/year)* 2 July 2004	Priority date *(day/month/year)* 4 July 2003

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[7] C07K 14/47, 19/00, A61K 38/17, A61P 31/04.

Applicant

THE NATIONAL UNIVERSITY OF SINGAPORE et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of **5** sheets, as follows:
      - [X] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

[X]	Box No. I	Basis of the report
[ ]	Box No. II	Priority
[ ]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[ ]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
[ ]	Box No. VI	Certain documents cited
[ ]	Box No. VII	Certain defects in the international application
[ ]	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
27 September 2004	25 May 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **FRANCES RODEN** Telephone No. (02) 6283 2239

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SG2004/000194

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1 (b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

☐ the international application as originally filed/furnished

☒ the description:

pages **1-49** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

☒ the claims:

pages as originally filed/furnished
pages* as amended (together with any statement) under Article 19
pages* **50-54** received by this Authority on **20 May 2005** with the letter of **17 May 2005**
pages* received by this Authority on with the letter of

☒ the drawings:

pages **1/4-4/4** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages
☐ the claims, Nos.
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to the sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages
☐ the claims, Nos.
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to the sequence listing (*specify*):

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SG2004/000194

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-35	YES
	Claims		NO
Inventive step (IS)	Claims	1-35	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-35	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents were cited in the Search Report:

D1 WO 2001/027289 A2.

D2 Journal of Chromatography, B, vol. 759, pages 237-246, J. L. DING et al.

D3 The Journal of Biological Chemistry, vol. 266, no. 10, pages 6554-6561, 1991, T. MUTA et al.

D4 Antimicrobial Agents and Chemotherapy, vol. 45, no. 10, pages 2820-2825, 2001, Y. H. YAU et al.

D5 The FASEB Journal, vol. 14, pages 1801-1813, 2000, N. S. TAN et al.

D6 Letters in Applied Microbiology, vol. 30, pages 161-166, 2000, J. M. MAURO et al.

D1 This citation teaches an S3 peptide tagged with a detectable label and a method for detecting LPS-containing bacteria using this labelled peptide. It also teaches the labelled peptide immobilized on a solid support and commercial use of such a peptide. See especially pages 9 line 12, page 10 line 11, examples 10 and 11 and claims 16, 17, 20, 21, 30 and 33. This document however does not teach or suggest a polypeptide comprising more than one S3 peptide and hence the present claims are novel and inventive in light of this document.

D2 discloses the synthetic peptide Sushi 3Δ conjugated to DADPA-immobilized agarose to form a novel affinity matrix that removes LPS from solutions. No suggestion is made in this document that more than one S3 peptide could be used in a polypeptide, therefore all claims are novel and inventive in light of this document.

D3 discloses the amino acid sequence of Factor C and the five repeating units called "Sushi" domains. This document teaches that Factor C binds LPS and acts as a "coagulation-complement factor." Isolation of the Sushi domains is not taught in this document and it represents general background art. All claims are novel and inventive in light of this citation.

D4 discloses four synthetic peptides based on the Sushi 1 and 3 regions of Factor C. These peptides and their mutants exhibit activities against 30 clinical isolates of *Pseudomonas aeruginosa*. It is shown that these peptides are tolerant of high-salt and adverse pH conditions which makes them promising therapeutic agents. There is no teaching or suggestion that Sushi 3 multimers could be produced, thus all claims are novel and inventive in light of this citation.

D5 teaches the isolation of three truncated fragments of Factor C, namely, sushi 123, sushi 1 and sushi 3. Sushi 1 and 3 are shown to each have a high-affinity LPS binding site. Two critical factors for the sensitivity of Factor C to LPS are identified, 1) the presence of multiple binding sites for LPS on a single Factor C molecule and 2) high positive cooperativity in LPS binding. Cooperative binding for sushi1 and non-cooperative binding for

(Continued on Supplemental Sheet)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SG2004/000194

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   [X] a sequence listing

   [ ] table(s) related to the sequence listing

   b. format of material

   [X] in written format

   [ ] in computer readable form

   c. time of filing/furnishing

   [X] contained in the international application as filed

   [ ] filed together with the international application in computer readable form

   [ ] furnished subsequently to this Authority for the purposes of search and/or examination

   [ ] received by this Authority as an amendment* on

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box V

sushi 3 was noted. The results show that in designing improved LPS binding and neutralizing peptides, the charge balance of the peptide is a critical parameter in addition to its structure. No teaching or suggestion is made that more than one sushi 3 peptide could be incorporated into a polypeptide giving enhanced LPS binding and removal. The claims are therefore novel and inventive in light of this document.

D6 discloses a strategy for the preparation of polypeptides containing multiple functional domains. Specifically disclosed is the construction of polymeric peptides in E.coli to prepare a library of plasmids coding for tandem repeats of the *Neurospora crassa* metallothionein gene. This document focuses on heavy metal binding and removal. There is no teaching or suggestion that this strategy could be useful for LPS-binding domains of factor C or specifically for the S3 peptide, in the treatment of sepsis or endotoxaemia. The claims are therefore novel and inventive in light of this citation.

All claims are industrially applicable.

PCT/SG2004/000194
Received 20 May 2005

WE CLAIM:

1. A polypeptide comprising more than one S3 peptides.

2. The polypeptide of claim 1 wherein the S3 peptides are in tandem repeat.

3. The polypeptide of claim 1 or 2 comprising 2 to 10 S3 peptides.

4. The polypeptide of claim 3 or 2 comprising two S3 peptides.

5. The polypeptide of claim 1 or 2 comprising three S3 peptides.

6. The polypeptide of claim 1 or 2 comprising four S3 peptides.

7. The polypeptide of claim 1 or 2 comprising eight S3 peptides.

8. The polypeptide of any one of claims 1-7 wherein at least two of the S3 peptides are separated by a linking sequence.

9. The polypeptide of claim 8 wherein at least one of the linking sequence is cleavable by protease.

10. The polypeptide of claim 8 wherein at least one of the linking sequence is cleavable by acid digestion.

11. The polypeptide of claim 10 wherein the at least one linking sequence comprises Asp-Pro.

12. The polypeptide of any one of claims 1-7 consisting of the S3 peptides.

13. The polypeptide of claim 6 which is rS3-4mer (SEQ ID NO: 9).

14. The polypeptide of any one of claims 1-13 tagged with a detectable label.

15. The polypeptide of claim 14 wherein the label is detectable by fluorescence.

16. DNA encoding the polypeptide of any one of claims 1-13.

17. An expression cassette comprising the DNA of claim 16.

18. A vector comprising the expression cassette of claim 17.

19. A host cell comprising the DNA of claim 16.

20. A method of producing a multimer of S3 peptide, comprising the step of expressing DNA encoding the polypeptide of any one of claims 1-13 in a host cell.

21. The method of claim 20 further comprising the step of isolating the polypeptide.

22. A method of producing a polypeptide having a desired number of S3 peptides, comprising the step of expressing in a host cell DNA encoding a polypeptide which comprises S3 peptides in greater number than the desired number, and wherein at least two of the S3 peptides are separated by a cleavable linking sequence; and subjecting the polypeptide to conditions suitable for cleaving the linking sequence to produce the polypeptide having the desired number of S3 peptides while keeping the S3 peptides intact.

23. The method of claim 22 further comprising the step of isolating the polypeptide having the desired number of S3 peptides.

24. The method of claim 22 or 23 wherein the conditions suitable for cleaving the linking sequence is acid digestion.

25. The method of claim 22 or 23 wherein the conditions suitable for cleaving the linking sequence comprises proteolytic digestion.

26. The method of any one of claims 22-25 wherein the desired number of S3 peptides is four; wherein the polypeptide encoded by the DNA comprises eight S3 peptides; and wherein the cleavable linking sequence occurs between the fourth and fifth S3 peptides in the polypeptide encoded by the DNA.

27. A method for detecting LPS-containing bacteria comprising the steps of contacting a sample to be tested for LPS-containing bacteria, with the polypeptide of any one of claims 1-13 and detecting binding between LPS and

PCT/SG2004/000194
Received 20 May 2005

the polypeptide.

28. A method for treating endotoxaemia or sepsis comprising the step of administering the polypeptide of any one of claims 1-13 to a patient suffering from endotoxaemia or sepsis.

29. A method for detecting LPS-containing bacteria comprising the step of contacting a sample containing LPS-containing bacteria with the polypeptide of claim 15 and detecting bacteria-associated fluorescence arising from the label.

30. The polypeptide of any one of claims 1-14 immobilized on a solid medium.

31. The polypeptide of claim 30 wherein the solid medium is agarose.

32. A method for removing LPS or LPS-containing bacteria from a sample, comprising the step of contacting the sample with the polypeptide of claim 30 or 31 under conditions which allow binding of LPS-containing bacteria to the polypeptide, and obtaining the unbound material which is substantially free of LPS or LPS-containing bacteria.

33. A commercial package comprising the polypeptide of any one of claims 1-13 and instructions for its use in detecting LPS-containing bacteria in a sample.

34. A commercial package comprising the polypeptide of any one of claims 1-13 and instructions for its use in treating endotoxaemia or sepsis.

PCT/SG2004/000194
Received 20 May 2005

35. A commercial package comprising the polypeptide of claim 30 or 31 and instructions for its use for removing LPS or LPS-containing bacteria from a sample.